STUDY QUESTIONS

1. Rank the three amino acids aspartic acid, tyrosine, and arginine in order of their affinities for a positively charged column at neutral pH.

2. A mixture of myoglobin (molecular weight 17,000 daltons), albumin (molecular weight 67,000 daltons), and catalase (molecular weight 220,000 daltons) is run on a gel filtration column. What is the expected order of elution of the proteins from the column?

3. Which of these proteins will bind to a negatively charged column at pH 5.0?

(a) pepsin (pI 1.0)

(b) ribonuclease A (pI 7.8)

(c) lysozyme (pI 11.0)

4. What is the primary method for assessing the purity of a protein during a purification? What type of protein contaminant in a purified preparation of a protein *will not* be detected by this method?

5. You are working on the purification of Protein X. From GenBank (a repository of vast amounts of protein and DNA sequence data), you ascertain that X has a molecular weight of 22,000 daltons. You run a gel filtration column on a crude cell lysate and, to your surprise, find that X runs as though it is a protein of 90,000 daltons. Puzzled, you start over, make a new cell lysate and run a cation exchange column (negatively charged column). You determine which fractions from the cation exchange column contain X and run one of these fractions over a gel filtration column. Now X runs according to your initial expectations (that is, like a protein of 22,000 daltons). How do you explain the initial gel filtration results, and why did the behavior of X change in the second running?

6. What are the objectives of the protein purifications in the three examples described at the end of the lecture?

ANSWERS TO STUDY QUESTIONS

1. Asp > Tyr > Arg

Aspartic acid has a net charge of –1 at neutral pH, and it will bind tightly to a positively charged column. Tyrosine has a net charge of 0; the attraction of the carboxyl group for the column will be partly countered by the repulsion of the amine for the column. Arginine will bind most weakly because it has a net charge of +1.

2. Catalase, followed by albumin, and last by myoglobin. The larger proteins elute first from a gel filtration column.

3. Lysozyme will bind tightly, because it will have a large positive charge at pH 5.0, and it will be attracted to the negatively charged column. Ribonuclease A will also bind, although more weakly, since it, too, will have a net positive charge at pH 5.0 (below its isoelectric point). However, pepsin will have a net negative charge (above its isoelectric point) and would not be expected to bind.

4. Denaturing (SDS) polyacrylamide gel electrophoresis is by far the most commonly used method. However, contaminants of the same molecular weight as the protein of interest cannot be detected. (In addition, contaminants other than proteins are not usually detectable.)

5. The best explanation is that Protein X is part of a complex with other proteins in the crude extract. This complex runs on the gel filtration column at the position of a 90,000-dalton protein. When the crude extract is fractionated on a cation exchange column, the other proteins that are associated with Protein X are purified away from it. Therefore, after this initial purification, Protein X run on a gel filtration column as expected. This type of behavior of proteins in typical in real purifications.

6. The objectives are: Angiostatin-to purify the protein sufficiently to allow unambiguous identification of the protein; Betaseron-to purify the protein away from any possibly toxic or harmful contaminants (in practice, this means purifying to homogeneity and testing for certain types of known toxins); creatine kinase-to separate the various isozymes of creatine kinase to allow assay of specific forms of creatine kinase.